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In This Issue

Microbial Source Tracking in South Carolina Surface Waters

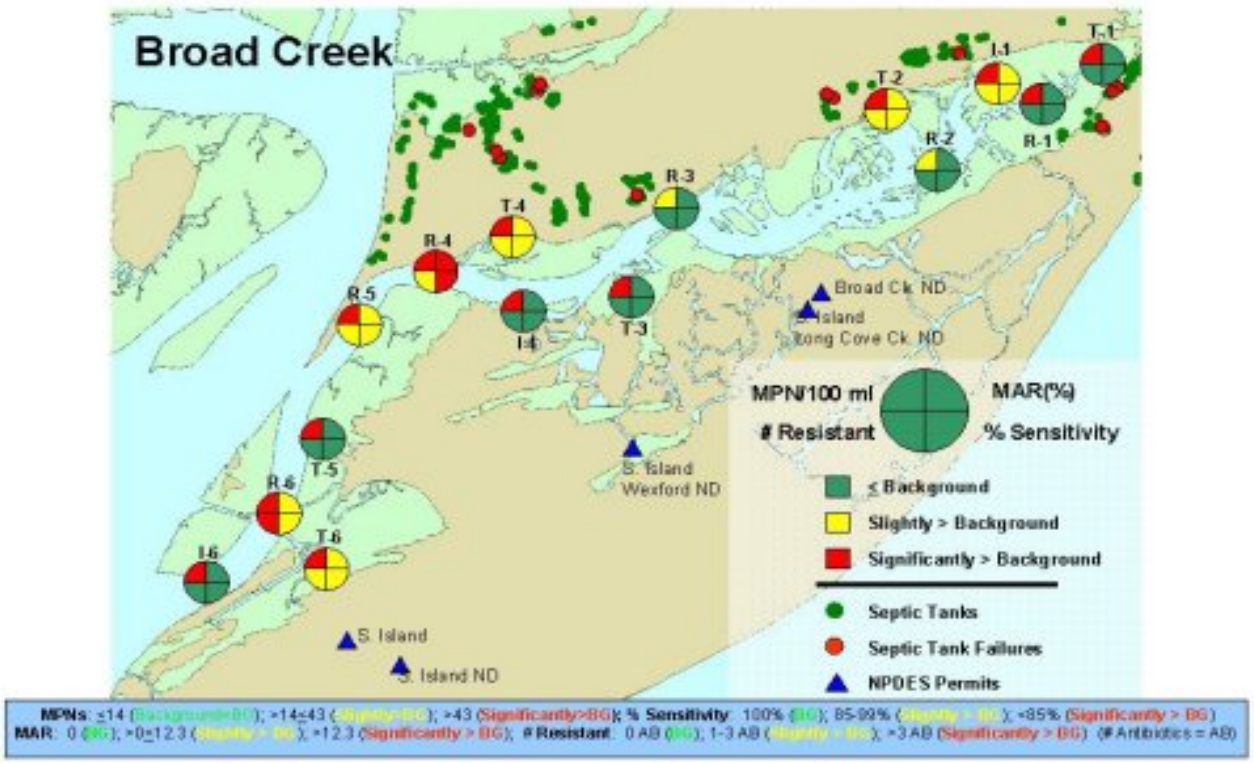
by Jill Stewart, Laura Webster, Brian Thompson, Jan Moore, and Geoff Scott

Society places a high value on coastal areas for living, working and recreating. There are approximately 66,645 miles of estuarine and coastal shoreline in the U.S. along which are some of the nation's most densely populated areas. More than half of the U.S. population (141 milion) live in coastal communities and the population is expected to increase 27 million by 2015 (USEPA 1999). Worldwide more than 55% of the population lives within 50 miles of the coast. Thirty-three of the 50 largest cities in the world are located in coastal areas as more than 80% of commerce in the global marketplace is ferried by maritime transportation (Dean,1997). The coastal zone area only represents 8% of the planet's surface but is the source of 26% of the world's primary productivity capacity (Dean, 1997).

One problem associated with this population influx into coastal areas is the increased amounts of human waste which may be produced. Generally human waste needs are met by either on-site waste disposal (e.g. septic tanks) or central sewers collection systems. One common practice in coastal areas is to land apply treated sewerage to golf courses as fertilizer to prevent direct discharge into coastal waterways. In addition, urbanization shrinks available wildlife habitat, resulting in more crowded wildlife populations. Evidence is mounting that both human and wildlife bacterial sources are contributing to the closure of shellfish harvesting areas in many regions of the U.S. Fecal contamination of environmental waters poses a serious threat to human health. Despite advanced sanitation conditions in the United States compared to other countries, contaminated waters continue to cause illness through drinking water use, recreational

water use and shellfish harvesting. Commonly used tools intended to protect the public from waterborne pathogens identify contaminated waters but cannot distinguish sources of contamination. This limitation makes it difficult to correct pollution problems and maintain clean watersheds in urbanized areas. In response, researchers are developing molecular and biochemical techniques for pollution source tracking. CCEHBR's Microbiology Program (Dr. Jan Moore, Jill Stewart, Laura Webster and Brian Thompson) in collaboration with the South Carolina Department of Health and Environmental Control, is engaged in a source tracking study in selected South Carolina watersheds which is utilizing three of the most promising methods for differentiating human and animal bacterial pollution sources. These methods include: Multiple Antibiotic Resistance (MAR) assays of Escherichia coli, ribotyping of E. coli and serotyping of F+RNA coliphages.

Multiple Antibiotic Resistance testing is a simple and powerful tool for learning more about the origins of microbial contaminants. Bacterial isolates are biotyped using Analytical Profile Indexing (API). Those confirmed as E. coli are tested for resistance to ten selected antibiotics. Bacteria from human sources are generally more resistant to antibiotics than bacteria from wild animal sources because humans more commonly undergo antibiotic therapy. Bacteria originating from domestic animals have intermediate MAR indices and different MAR patterns than those from humans. Prior to the DHEC source tracking study, NOAA microbiologists had determined the resistance patterns of E. coli isolates from various surface waters, eight wastewater treatment facilities, a septic tank, 11 humans and 39 animals. Some notable results include the comparison of a developed and undeveloped estuary. Seven out of 15 (47%) surface water sites in the developed watershed had E. coli strains that were resistant to one or more antibiotics. Only 3 out of 15 (20%) sites in the undeveloped estuary contained resistant strains, 1 site of which was located near a spray field for a wastewater treatment facility. Overall, MAR in South Carolina averaged 14% in wastewater treatment plants versus 3% in urban watershed and less than 1% in undeveloped, pristine watersheds. Studies in other regions of the country (e.g. Florida and Maryland) indicate higher MAR of 9-25% in urban areas and 3-11% in rural areas. These regional differences may be related in part to differences in tidal range as South Carolina has a mesotidal tidal range that would be 3-6 times greater than in these other microtidal regions. When normalized for these tidal range differences, the MAR rates are quite similar throughout coastal waters of the U.S.



Coliform bacteria and multiple antibiotic resistance in Broad Creek. Septic tanks (green and red dots) and NPDES permitted sites (blue triangles) indicate a possible correlation of MAR results with waste management.

Broad Creek Map

Ribotyping is a molecular procedure which identifies unique sequences within E. coli ribosomal DNA. The technology enables researchers to identify the culprit of contamination, much as human DNA analysis or fingerprinting is used by law enforcement. Bacterial DNA is isolated and digested with a restriction enzyme that cleaves the nucleic acid at a specific sequence. The resulting DNA fragments are separated by electrophoresis in an agarose gel, transferred to a nylon membrane, and hybridized with a labeled probe. The label enables the DNA banding pattern to be visualized, and sizes are assigned to each band according to a molecular weight marker. The resulting banding pattern is compared to those of reference samples from the same vicinity (chickens, cows, pigs, domestic wastewater, etc.) and each environmental *E. coli* isolate is assigned an origin. The analysis process is facilitated by a database administered at the University of Florida.

Coliphages are viral indicators of enteric pathogens in environmental samples. The F+RNA (Leviviridae) family of coliphages can distinguish between human and animal waste contamination by typing isolates into one of four subgroups using genetic hybridization or serological methods. Groups I and IV are generally indicative of animal feces whereas groups II and III are more human-specific. This technology has only been applied in a few locations worldwide, so hundreds of South Carolina isolates will be compared to reference samples to check for variations in coliphage distributions.

The current surface water project is expected to be completed in the spring of 2001. Preliminary analyses suggest that MAR, ribotyping and coliphage typing differ in their sensitivity and ability to track fecal pollution sources. It is likely that a combination of source tracking procedures will emerge as the best approach to addressing watershed clean-up efforts.

References

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Fipronil - New Insecticide to be Studied

by Pete Key

Fipronil is a fairly new insecticide first introduced commercially in 1993. It acts by blocking the chloride channels at the gamma-aminobutyric acid receptor in the central nervous system. This leads to neural excitation and eventually death of the exposed organism. The importance of this insecticide has been gradually increasing and is set to gain even wider use with the recent ban of chlorpyrifos (Dursban). Fipronil can now be found as the active ingredient of Frontline (to kill fleas and ticks on pets), Combat and Maxforce (to kill roaches), Termidor (to control termites), Regent and Icon (to control crop pests) and Chipco (to control turf insects on golf courses and lawns). It is actively marketed to control crop pests in many countries. Due to fipronil being a recent addition to the pest war arsenal, not much is known of its toxic effects to non target species. This gives the Marine Ecotoxicology Branch a great opportunity to fill in the gap on fipronil toxicity and chemistry. Using the grass shrimp as a model marine invertebrate, we will perform acute and chronic toxicity tests to determine sensitivity to this insecticide on both adults and larvae. Based on these results, complete life cycle studies can also be performed. The mummichog will be used as the model marine vertebrate with similar tests being performed. To complete the picture, the Ecotox chemistry group will determine the recovery of fipronil and its metabolites in laboratory samples and how much (if any) is found in environmental samples. While no insecticide will be safe to all non-target organisms, our data can help regulators determine when and where an insecticide can best be applied.

Louisiana Dolphin Rescue

by Eric Zolman

Earlier this summer, a small group of bottlenose dolphins (Tursiops truncatus) were trapped in a canal in the Rockefeller Wildlife Refuge near Grand Chenier, Louisiana. The dolphins had entered the canal through a breach in the levy. The breach had been created in order to move a dredge barge into the canal from an adjoining waterway. The breach remained during dredging, allowing animals (including dolphins) to freely move between the adjacent canals. At the completion of the dredging activities, the levy was resealed. During the resealing, the laborers became aware that there were four dolphins still in the dead-end canal; however, the dredge crew believed that the dolphins would be able to exit the canal through a spill-way gate. Refuge personnel were informed of the situation and monitored the status of the

dolphins. It soon became apparent to the state personnel that the dolphins were unable or unwilling to exit the canal via the spillway.

Due to an abundance of prey and the high salinity in the canal, the dolphins were not in any immediate danger, there was concern though, that with the onset of colder temperatures, fish would become scarce, thereby placing the dolphins at risk of starvation. In response to these concerns, a rescue operation was planned by the NMFS-SEFSC stranding coordinator, Blair Mase. This effort included personnel from the NMFS-Miami, the NMFS-Pascagoula, the NOS/CCEHBR, the Texas Marine Mammal Stranding Network and Rockefeller Wildlife Refuge employees as well as an experienced marine mammal veterinarian.

The rescue team arrived at the Refuge on Monday, August 7, and made a preliminary reconnaissance of the site. The canal was approximately three miles long, roughly fifty feet across and was bordered by steep, muddy banks. Having been recently dredged, the canal was deep, ranging from eight to twenty feet along most of its length. At one end, the canal dead-ended while at the opposite end, there was a pool, roughly sixty feet in diameter. A concrete spillway separated this pool from the adjacent canal, which ran to the open waters of the Gulf of Mexico. During this initial visit, three of the dolphins were observed actively feeding on fish entrained in the current running through the spillway. Equipment was staged along the banks of the canal and the remainder of that evening was spent planning for the rescue efforts.

Initially, the plan was to wait for the dolphins to return to the pool, stretch a net across the pool mouth and gradually purse it around the dolphins. During this process, the dolphins would be induced to strike the net, then be restrained by members of the rescue team and lifted from the pool by stretcher for a brief physical examination before being released into the adjacent canal. No dolphins were to be seen near the spillway after the team's arrival the morning of the Eighth. A boat was dispatched to survey the canal and soon all four dolphins, three adults and an older calf, were located over a mile away from the spillway. It soon became apparent that the dolphins were not inclined to visit the pool, where the team was lying in ambush.

Plan B called for the dolphins to be driven toward, and then into, the pool. This was accomplished with the help of a hookelau, stretched between two jon-boats across the width of the canal. The hookelau consisted of a long, horizontal rope with weighted lines spaced at regular intervals along it. Although similar devices had been used to herd pelagic dolphins, most of the rescue team were skeptical as to whether coastal bottlenose dolphins would pay any heed to this flimsy ‘barrier'. To the crew's surprise though, the dolphins were soon moving toward the pool, with the hookelau 70-80 feet behind them.

After the dolphins swam into the pool, the net was to be pulled across it's mouth with a rope stretched between the canal's banks. The tension level increased as the dolphins were seen edging closer and closer to the pool. One of the dolphins managed to swim past the hookelau back into the canal, but the other three gradually surfaced closer and closer to the pool. The dolphins milled in the water beneath the taut rope for some time before moving into the pool. As soon as the three dolphins were seen to surface within the pool, the net was quickly pulled across the pool's mouth, effectively barring the dolphin's escape back into the canal. After a few aborted charges towards the net, the dolphins adopted a wait-and-see posture, content to remain submerged within the pool. The net was slowly worked around the edges of the pool, gradually decreasing the area available to the dolphins. During this pursing of the net, the calf managed to slip beneath the net's lead line, leaving two adult dolphins in the shrinking net bag.

Dolphins ‘fleeing' from the hookelau

After the calf's escape, the remainder of the day's work went according to plan and the team soon had both dolphins restrained. Both dolphins appeared thin but otherwise in good health. The first dolphin to be examined was believed to be the mother of the escaped calf. However, she wasn't lactating and, given the size of the calf, there was little concern over the pairs forced separation. Blood and a blubber biopsy were collected from both dolphins and both were tagged and freeze-branded (‘71' for the mother and ‘70' for the second dolphin, a male) to enable them to be recognized in the future. Both were released separately and without incident and the team returned to the wildlife center to rest up for the next day's efforts.

The following day, well-rested and with the previous day's experience, the capture of the remaining two dolphins proceeded smoothly. The calf had remained near the spillway end of the canal overnight. The fourth dolphin was soon located and herded toward that end. Both dolphins were soon ushered into the pool and the net was again strung across the canal. Unlike the previous day, neither dolphin charged the net, both remaining relatively quiescent, content to stay submerged, for the most part, in the pool. The net was again worked around the pool's edges and both dolphins were soon being restrained by members of the rescue team. The calf turned out to be a female while the larger dolphin was a male. These two also appeared thin but otherwise healthy, similar to the pair the previous day. After their brief physical exams and tagging, both dolphins were on their way to the freedom of open water.

The Case of the Elusive Sperm Whale Oil

by Julie Carter

The part of my job that I enjoy the most can also be the most frustrating! When Forensics receives a case, the first thing we do is look to see if we have any comparison standards. If not, the search begins. We start with the obvious first. If it's a marine mammal sample we're looking for, we ask our marine mammal experts here in-house. If they don't have any, they still can help by providing names of colleagues who may have a sample.

For one case involving two molluscan species, we started with the Smithsonian in Washington, DC. They sent me a sample of one species and suggested I try The Smithsonian Tropical Research Institute in

Balboa, Panama, for the other. I've offered to go get it, but I think I'm just going to have to wait on them to mail it to me.

Another case involves sperm whale oil. I've learned a lot about sperm whales from this assignment. Whales are protected under the Marine Mammal Protection Act (MMPA) of 1972 so the main sources of legal samples are pre-1972 samples from museums and samples from marine mammal stranding networks. I have called every whaling museum and marine mammal stranding coordinator. They have suggested more museums and marine mammal people to call. I've gotten a couple of samples, but we need more so I'm still in hot pursuit.

But, of course, finding the standards is only half the battle. Getting people to call back and/or actually send the stuff is the fun part. There's a fine line between being persistent and nagging.

Another part of my job is making sure we have the permits we need for all of these samples. It's a jungle (of paperwork) out there. If we receive samples from permitted researchers within the U.S., we are covered under their permit. However, if we want to import a sample from a protected or an endangered species, we must first get a permit from the agency that has jurisdiction over that species (like NMFS for most marine mammals) and then a Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) permit from U.S. Fish and Wildlife. We were told to allow up to 6 months for a NMFS permit and 60 to 90 days after that for a CITES permit. So you can see that it is definitely worthwhile to find a U.S. source if at all possible.

The least interesting part of my job is also the most important. As the evidence custodian for the Forensics Branch, I receive evidence from law enforcement agents from many different federal and state agencies. It can be anything from a 100 lb. smelly dead sea turtle in a large locked tool box to a single frozen turtle egg in a ziplock bag. I know which one I prefer but I don't get to choose - I get whatever the agent wants to send. It is my responsibility to make sure that the evidence is kept safe and secure and that there is a traceable paper trail documenting the chain of custody.

After we send the final report for a case to the agent, I contact the agent to let him or her know I am shipping back the evidence. Sometimes, for whatever reason, the agent doesn't want the evidence back. In that case I send a release of evidence form. After the agent returns the signed release of evidence form, the evidence is either destroyed (if we already have enough of those type of samples in our archive) or given to Elizabeth Bowen, our archivist, to log into our archive for use in research.

The Forensics Archive

by Elizabeth Bowen

In the Forensics Branch, one of our most important tools (aside from the employees, of course) is our archives. Although samples in the archive originate from many different places, many come to me by way of Julie Carter, our evidence custodian. Once a case is closed, or we are no longer working on it, it is Julie's job to contact the law enforcement agent that originally sent us the evidence. She either sends the evidence back to the agent or requests them to release the evidence to her custody.

Most often, the enforcement agents do not want the evidence returned to them (usually the large, smelly samples). From that point, it joins the vast collection of marine tissues we have in our forensic archive.

The collection is comprised of tissues from marine mammals, fish, reptiles, and some shellfish. Included in this collection are manatees, sharks, sea turtles, lobsters, conch, and tunas- just to name a few. To date our archive contains over 9,000 samples from about 150 different species and it's growing every day!

In addition to containing many different species, the archive also contains many different types of tissue samples, ranging from muscle tissue to organs to whole carcasses (which are, of course, our personal favorite). We even have a few hair samples in our database. We have sea turtle eggs and shells, as well as hair combs and jewelry, lotions, soups, even boots- all made from sea turtle tissues. Some of these products are made based on the belief that sea turtle eggs are an aphrodisiac, others, because of the beauty of the shell or sweetness of the turtle meat itself. Just recently, we received over 1,000 sturgeon barbels from the SCDNR, which may help us learn more about this endangered species. We also receive bluefin tuna samples from all over the world.

The samples in our archive serve many different purposes for many different organizations worldwide, such as population, genetic, and impact studies. Our samples may also be used to test and develop new methods of species identification. Researchers within our own lab, as well as those outside the lab, request samples from our archive. The Forensics Branch is currently sending bluefin tuna vertebra to a researcher in Miami doing population studies on fish in the Gulf of Mexico and the Atlantic Ocean. We also provide samples to graduate students in the area working on their thesis.

We are constantly looking for new and different species to house in our archive. In building a database of diverse samples that can provide viable DNA to scientists worldwide, we hope to further the preservation and protection of marine organisms, as well as help provide the tools to punish those that work against these goals. Although sometimes tedious (logging in all 1,000 plus samples of sturgeon), the reward of actually obtaining a rare or sought after sample is well worth all the effort.

Upcoming Events

October 5 - CCEHBR General Staff Meeting: Lois Winemiller Auditorium at 10:00 AM

October 6 - Fort Johnson Seminar Series: Marine Genomics: The Ecology of the Cell or the Theory and Practice of Everything presented by Bob Chapman, SC Dept. Natural Resources (held in the MRRI Auditorium at 4:00 PM)

October 9-12 - Media Relations training: A member a the management staff will attend this class in Shepardstown, WV

October 10 - Adopt a Highway: CCEHBR volunteers should meet at the James Island Cinema parking lot at 9:00 AM to take part in litter removal from Central Park Avenue.

October 12 - Federal Executive Board Meeting: held at noon in the Lois Winemiller Auditorium

October 18-20 - CDC National Pfiesteria Conference: held in Atlanta, Ga, and attended by members of the Coastal Research Branch

October 20 - Fort Johnson Seminar Series: TBA presented by A. P. "Hap" Wheeler, Clemson University (held in the NOAA Auditorium at 4:00 PM)

October 25 - CCEHBR Seminar Series: Production and Use of Geographic Data in Environmental Modeling and Statistical Analysis by Chris Nichols, Tom Siewicki, Robin Puett; at noon in the Lois Winemiller Auditorium

October 27 - Fort Johnson Seminar Series: TBA presented by David White, Univ. of Tennessee

November 1-17 - US-Japan Shellfish Conference and the 29th Joint Meeting of US-Japan National Resource Aquaculture Panel: members of the Cooperative Oxford Laboratory will be attending this meeting sponsored by the Japanese National Institute of Aquaculture.

November 2-3 - Right Whale Implementation Team Meeting: Wayne McFee to attend in Jacksonville, FL

November 3 - Fort Johnson Seminar Series: TBA presented by Joe Pawlik, UNC Wilmington

November 5-10 - The 24th Annual Larval Fish Conference: a member of the Forensics Branch will attend in Gulf Shores, AL

November 8-11 - AISES Conference: A member of the Marine Ecotoxicology Branch will participate on a panel discussion in Portland, OR

November 10 - Fort Johnson Seminar Series: Myoglobin Expression in Antarctic Icefishes: "Now You See It, Now You Don't" presented by Bruce Sidell, University of Maine (held in the MRRI Auditorium at 4:00 PM)

November 12-16 - SETAC: members of CCEHBR and NIST will be attending this meeting in Nashville, TN.

November 15-18 - 4th International Conference on Shellfish Restoration Hilton Head, SC: NOS is a sponsor of the ICSR and serves on the steering and planning committees of this conference. Additionally, a poster will be presented on the NOS Shellfish Information Management System (SIMS) and a developing shellfish restoration theme in SIMS.

December 1 - Fort Johnson Seminar Series: Stable isotopic records of nutrient utilization and upper water-column properties in the Gulf of California presented by Carol Pride, University of Charleston (held in the MRRI Auditorium at 4:00 PM)

December 4-9 - WHOI Symposium on Harmful Marine Algae in the US - Japan: a member of the Lab will serve on the oyster disease panel

December 8 - Fort Johnson Seminar Series: TBA presented by Margie Peden-Adams, MUSC

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